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Abstract

Background: The relationship between maternal selenium (Se) status and child neurodevelopment has been scarcely assessed. In a previous study we observed an inverse U-shaped association between maternal Se concentrations and infant neurodevelopment at 12 months of age. In this study, this non-linear association was explored at preschool age. The effect modification by breastfeeding, child's sex and cord blood mercury was also evaluated.

Methods: Study subjects were 490 mother-child pairs from the Spanish Childhood and Environment Project (INMA, 2003-2012). Child neuropsychological development was assessed at around 5 years of age by the McCarthy Scales of Children's Abilities (MSCA). Sociodemographic and dietary characteristics were collected by questionnaire at the first and third trimester of gestation and at 5 years of age. Se was measured in serum samples by ICP-MS at the end of the first trimester of pregnancy (mean ± standard deviation (SD) = 12.4 ± 0.6 weeks of gestation).

Results: The mean ± SD of maternal serum Se concentrations was 79.9 ± 8.1 μg/L. In multivariate analysis, no linear association was found between Se concentrations and the nine MSCA scales. Generalized additive models indicated inverted U-shaped relationships between Se concentrations and the verbal and global memory scales. When assessing the influence of effect modifiers, breastfeeding played a role: the association between Se and neuropsychological development was inverted U-shaped for the quantitative, general cognitive, working memory, fine motor, global motor and executive function scales only for non-breastfed children.

Conclusion: Low and high maternal Se concentrations seem to be harmful for child neuropsychological development, however further studies should explore this non-linear relationship.

Keywords: Selenium; Pregnancy; Children; Neurodevelopment; Nutrient

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1. Introduction

Selenium (Se) is a trace element that is essential for early development since it is incorporated into selenoproteins with a crucial role in preventing damage from oxidative stress during foetal development (Mihailovič et al., 2000; Mistry and Williams, 2011). During pregnancy there is an enhanced demand for Se since it is in part transferred to the foetus (Nandakumaran et al., 2003; Santos et al., 2017); in fact, a decreasing concentration of Se in maternal blood has been reported during this period (Pieczyńska and Grajeta, 2015).

A deficiency of Se during pregnancy has been associated with adverse outcomes, which include miscarriages, preeclampsia, gestational diabetes, premature rupture of membranes and intra-uterine growth restriction (Mistry et al., 2012). In addition, selenoproteins are important for normal brain function, and a lower expression of them can lead to impaired cognitive function and neurological disorders (Pillai et al., 2014). However, adverse effects in the nervous system due to a chronic overexposure to Se have also been identified in China, USA or Italy in populations living in high Se content areas (Vinceti et al., 2014).

Se is also present in breast milk as organic compounds and selenoproteins, especially the GPx. In fact, human milk is fundamental for the infant's optimum Se status; thus, a higher plasma Se concentration and glutathione peroxidases (GPx) activity in breastfed infants compared with formula-fed or cow milk-fed infants has been reported (Dorea, 2002). In addition, breast milk has other nutrients, such as fatty acids, that are crucial for development of the nervous system (Innis, 2014).

Very few prospective studies have evaluated the relationship between Se status during pregnancy and child neuropsychological development among populations with

intermediate Se levels and the results obtained have been heterogeneous. Thus, positive, negative and null effects on child neuropsychological development have been observed in relationship to prenatal Se levels (Amorós et al., 2018; Kippler et al., 2016; Oken et al., 2016; Polanska et al., 2016; Skröder et al., 2015; Yang et al., 2013) and, in addition, the shape of this association has scarcely been assessed.

In a previous study, we reported an inverted U-shaped relationship between maternal Se status and child neuropsychological development evaluated at around 12 months of age, the turning point for this association being estimated at 86 µg/L (Amorós et al., 2018). The relevance of these findings justifies a further evaluation of Se-related effects at older ages.

The aim of this study was to evaluate the association between maternal Se status and child neuropsychological development evaluated at 5 years of age in the same Spanish population. We also assessed the effect modification by children's sex, breastfeeding, and cord blood mercury.

2. Methods

2.1 Study population

Study subjects were participants in the Valencia region cohort of the INMA Project (Childhood and Environment Project: http://www.proyectoinma.org) – a multicentre birth cohort study that aims to investigate the effects of environmental exposures and diet during pregnancy on foetal and child health in different areas of Spain.

The study protocol has been reported elsewhere (Guxens et al., 2012). Briefly, pregnant women were recruited at the beginning of their pregnancy in the Spanish region of Valencia (n=855, 2003-2005). A total sample of 787 (92%) women was followed up until delivery. Their children were enrolled at birth and monitored until 5 years of age (n

=536, 63%). The final study population was made up of 490 (57%) mother–child pairs for whom Se concentrations and neuropsychological test scores were available. Informed consent was obtained from all participants in each phase and the study was approved by the Ethics Committee of La Fe Hospital, Valencia, Spain.

2.2 Selenium concentrations

Concentrations of Se were determined in serum samples taken at the end of the first trimester of pregnancy (mean ± standard deviation (SD) = 12.4 ± 0.6 weeks of gestation). The concentrations of serum Se were determined by inductively coupled plasma mass spectrometry with the collision/reaction cell system in hydrogen mode. More information about the methodology used for Se analysis has been reported in detail elsewhere (Amoros et al., 2018) and can be found in the supplementary material. The limit of detection was 0.03 µg/L and no samples had concentrations below this value. Se concentrations were corrected according to the variations in three daily measures of the Seronorm™ (lot MI0181) reference material. The correction was performed by adding to each measure the difference between the daily mean of the reference measures and the overall mean of the reference measures (Amorós et al., 2018).

2.3 Child neurodevelopment evaluation

The neuropsychological development of the children was assessed at 5 years of age (mean ± SD = 5.8 ± 0.16 years) by using a standardized version of the McCarthy Scales of Children's Abilities (MSCA) adapted to the Spanish population (McCarthy D, 2009). The MSCA comprise 18 subtests that yield standardized test scores for six conventional domains. The verbal scale refers to cognitive tasks related to the processing of verbal

information; the perceptual-performance scale refers to cognitive tasks related to perceptual information processing, including manual performance; the quantitative scale assesses numerical abilities; the global memory scale considers short-term retention of information (verbal, visual or numerical); and the motor scale refers to fine (e.g. drawing) and gross (e.g. balance or accuracy) abilities. The sum of the first three scales provides a general cognitive scale. MCSA's subtests were reorganized into new outcome subarea scores according to those tasks highly associated with a specific neurocognitive function (Julvez et al., 2007, 2011). The new outcome subareas were: working memory, which refers to those cognitive tasks related to temporarily storing and managing the information required to carry out other cognitive tasks such as learning, reasoning and comprehension; executive function, which refers to those cognitive tasks critical to non-routine, goal-oriented situations that are performed by the pre-frontal cortex; and fine motor. Items comprising each of the scales are indicated in Figure S.1 of the Supplemental Material. Testing was conducted by two psychologists using a strict protocol.

2.4 Other variables

The women completed two questionnaires during their pregnancy, one at the first trimester (mean ± SD) = 12.6 ± 1.4 weeks of gestation) and the other at the third trimester (mean ± SD = 32.3 ± 1.8 weeks of gestation). The questionnaires were administered by trained interviewers and focused on sociodemographic, dietary, environmental and lifestyle information during pregnancy. The maternal covariates and potential confounders collected were: country of birth, age, body mass index before pregnancy, level of education, parity, area of residence, working status during

pregnancy, smoking at the beginning of pregnancy and season of sampling. We also obtained data on paternal age, working status and level of education.

Parental social class was defined from the maternal or paternal occupation during pregnancy with the highest social class, according to a widely used Spanish adaptation of the International Standard Classification of Occupations, approved in 1988 (ISCO88) (Class I+II: managerial jobs, senior technical staff and commercial managers; class III: skilled non-manual workers; and class IV+V: manual and unskilled workers).

Information on diet during pregnancy was collected by using a semiquantitative food frequency questionnaire (FFQ). Maternal intake of bread, eggs and seafood were identified as sources of Se in our study population (Amorós et al., 2018), and for this reason they were tested as possible confounders in the present study.

Information on the children's gestational age, sex, birth weight, breastfeeding (no, yes) and birth size was obtained from clinical records and subsequent interviews. Breastfeeding was defined as receiving breast milk, although it could be supplemented with any other food or liquid, including non-human milk.

Information about maternal and paternal working status, maternal and paternal smoking habit in the presence of the child, and a proxy of the maternal verbal intelligence quotient (IQ) was obtained in an interview at the same time-point as the neuropsychological development assessment. The maternal verbal IQ proxy was assessed using the Similarities Subtest of the Weschler Adult Intelligence-Third Edition (WAIS-III).

Cord blood total mercury (THg) was determined by thermal decomposition, amalgamation and atomic absorption spectrometry using a single-purpose AMA-254 advanced mercury analyser (LECO Corporation, St. Joseph, Michigan, USA). We

categorized this variable (<15 vs ≥15 μg/L) according to the equivalent for the WHO Provisional Tolerable Weekly Intake (1.6 μg/kg of body weight per week).

2.5 Statistical analysis

Multivariate linear regression models were built through a two-step procedure to assess the relationship between Se concentrations and the different scales of the MSCA. In the first step, a core model was built with the raw scores of each scale as the response variable and parental and child sociodemographic variables as possible covariates, adjusting in all cases for psychologist, sex and age at the time the test was administered. These multivariate models were built following a backward elimination procedure, using all variables with a p-value < 0.2 in the univariate models as candidate covariates and retaining those with a p-value < 0.1 in the likelihood ratio test (LRT). The Se concentrations were then introduced into these adjusted models. In the second step, additional confounders were included if they changed the magnitude of the main effect in a significant way with a 5% significance level (Lee, 2014). The potential confounder variables were those with evidence of being determinants of the maternal Se status in a previous work (Amorós et al., 2018), namely, maternal country of birth, age, smoking at the beginning of pregnancy, season of sampling and intake of seafood, eggs and bread. Generalized additive models (GAM) using natural cubic smoothing splines with one internal knot were employed to assess the linearity of the relationship between child neuropsychological development and Se concentrations by graphical observation and the Akaike information criterion (AIC). Segmented models were used to determine a breakpoint in the linear association between development scales and Se concentrations. Heuristic algorithms were used to find the potential location of one or several break points of the segmented regressions (Muggeo, 2003). Multivariate linear models using

only data below or above the breakpoint were checked. Effect modifications (interaction) by sex of the child, breastfeeding, and cord blood THg were assessed. To do so, the interaction effect of these variables with Se concentration was tested using the LRT for the linear model, and AIC scores were compared for the GAM models with and without interaction. All the analyses were performed using R, version 3.3.0, software. R packages mgcv, segmented and ggplot2 were used to implement the GAM and segmented models, and to plot the graphs, respectively.

3. Results

Parental and child characteristics are shown in Table 1. Ninety-three per cent of the women were born in Spain, 28% had finished university studies, and 45% of them belonged to the lowest social class. The mean ± SD Se concentration was 79.12 ± 8.02 μg/L. The geometric mean (SD) THg in cord blood was 10.60 (11.11) μg/L.

The variables included in the multivariate models for each scale can be found in Figure S.2 of the Supplemental Material. The associations between maternal Se concentrations and the 9 MSCA scales considered were inverse in all cases (Table 2), although the p-values of the multivariate models were all >0.05.

Non-linear multivariate GAMs showed a fit improvement (lower AIC score) for the association between maternal Se concentrations and children's verbal and global memory scales, with respect to the multivariate linear models and to the same multivariate linear models without including Se as an explanatory variable – that is, the baseline model which only includes the core model and the potential confounders. The AIC scores for the GAM, the multivariate linear model and the multivariate linear model without Se were 3645.8, 3650.6 and 3649.4 for the verbal score and 3109.3, 3110.8 and 3110.3 for the global memory score, respectively. Direct observation of the

estimated splines of the GAM showed associations with a range from inverted U to horizontal shapes for the 9 different scales, with the clearest inverted U-shapes for the verbal, general cognitive, global memory and executive function scales (Figure 1). Segmented regression with one breakpoint estimated the change point at 83.7 µg/L (95% CI= (77.2, 90.1)) for the verbal score and at 85.6 µg/L (95% CI= (77.4, 93.9)) for the global memory scale.

Among the potential effect modifiers (sex, breastfeeding, and cord blood THg), only interactions with breastfeeding showed effect modifications (linear interaction p-values shown in Table 2). An improvement in terms of AIC was found on adding the interaction term to the GAMs in the quantitative, general cognitive, working memory, fine motor, global motor and executive function scales, resulting in inverted U-shaped associations with Se for the non-breastfed children and flat or linear and slightly descendent associations for the breastfed children (Figure 2). We also evaluated the effect modification associated with the time of breastfeeding (None, 0-16, >16 weeks) and time of exclusive breastfeeding (None, 0-16, >16 weeks) but we obtained similar results for the non-breastfed infants. The other two categories overlapped (data not shown). The GAMs for the interaction with sex and cord blood THg are shown in Figures S.3 of the Supplemental Material and Figure 3, respectively.

4. Discussion

In this birth cohort study, we observed an inverted U-shape relationship between maternal Se status and some domains of child neuropsychological development assessed at around 5 years of age. This shape was statistically relevant for the verbal and global memory scales, and a monotonically descending relationship between these scales and Se concentrations was observed in children whose mothers had Se serum concentrations

above approximately 85 μg/L at the first trimester of pregnancy. These results are in line with those we showed in a previous study on the same cohort, where neuropsychological development was assessed at around 12 months of age, and a statistically relevant inverted U-shaped relationship was observed with the mental scale measured with the Bayley Scales of Infant Development (Amorós et al., 2018). Previous studies have evaluated the association between prenatal Se concentrations and child neuropsychological development, showing somewhat conflicting results. Positive associations were found between prenatal Se and psychomotor and language development in children from Bangladesh (median of Se in haemoglobin at 30 weeks of gestation: 0.46 μg/g) (Skröder et al., 2015) and Poland (mean of Se in serum at the first trimester of pregnancy: 48.3 μg/L) (Polanska et al., 2016, 2017). Additionally, a positive, but only marginally significant, association was observed between cord blood Se serum (geometric mean of 40.1 ng/g) and the language domain among children who were non-carriers of the ε4 allele for the *apolipoprotein E* gene, but not in the ε4 carriers (Snoj Tratnik et al., 2017). This ε4 allele was also found to be a modifier of the association between mercury and cognitive performance in the same population, the carrier children being the ones who obtained poorer scores. However, other studies failed to find any significant association with these and other assessed aspects of neuropsychological development in children from Greece (Kippler et al., 2016), USA (Oken et al., 2016), Poland (Polanska et al., 2016) and Japan (Tatsuta et al., 2017). Se levels (mean ± standard deviation) observed in these studies were 23 ± 8.6 μg/L in maternal urine at the first trimester of pregnancy in Greece (Kippler et al., 2016), 205.6 ± 34.6 ng/mL in erythrocytes at mid-pregnancy in USA (Oken et al., 2016), and 66.3 ± 10.2 and 67.0 ± 9.6 ng/g (for boys and girls, respectively) in cord plasma in Japan (Tatsuta et al., 2017). Conversely, Saint-Amour et al. (2006) observed that prenatal Se

levels were associated with alterations in the visual evoked potentials of Inuit children (cord blood Se was 4.44 ± 2.08 μmol/L) (Saint-Amour et al., 2006). However, none of these studies explored the shape of the relationship between prenatal Se levels and the children's neuropsychological outcomes. Some evidence of a non-linear shape has been observed in very few studies. For example, Yang et al. (2013) reported an inverted U-shaped relationship between cord blood Se (median cord serum was 63.1 μg/L) and scores for behaviour assessment at 3 days of age in a cohort in China (Yang et al., 2013), Ode et al. (2015) observed a higher likelihood of being diagnosed with attention deficit and hyperactivity disorder among children with Se levels above the 95th percentile (59 μg/L) (Ode et al., 2015) and Varsi et al. (2017) observed a negative association between prenatal Se concentrations <0.90 μmoles/L and infant neurodevelopment (equivalent to 71 μg/L) (Varsi et al., 2017).

In the present study we have also observed a differentiated relationship between maternal Se levels and child neuropsychological development according to breastfeeding, the non-breastfed children being the ones for whom the detrimental effect of extreme, mostly low, levels of Se was seen. This seemingly protective effect of breastfeeding was most clearly observed at low values of maternal Se levels, thereby suggesting that breastfeeding could offset the low Se levels during the prenatal period. Se is able to cross the mammary gland and it is transferred from mother to child; in fact, the amount of Se in maternal milk and its bioavailability is higher compared with cows' milk formula, which causes higher Se levels in breastfed compared to non-breastfed children (Dorea, 2002). In any case, the amount of Se present in breast milk has been observed to be different depending on where the mother lives, because of the different Se contents in the soil, which affects Se accumulation in the cereals consumed by

animals and humans (Zachara and Pilecki, 2000). Therefore, the impact of lactation could vary among countries.

One limitation in our study is the unavailability of Se speciation data; the inverse association observed in our cohort could be related to one or several of the Se forms present in the body. This is a concern of crucial importance for further epidemiological studies on this topic. Another limitation could be that the associations at the tails of Se could be somewhat weak due to the small number of individuals with very low and very high levels. However, sensitivity analyses excluding those individuals with Se levels < quantile 0.025 (66.9 μg/L) or > quantile 0.975 (97.4 μg/L) showed similar results. The results for the modifying effect by breastfeeding should be interpreted with caution because we did not take into account some factors that could be influencing the children's selenium status, such as time of weaning or the amount of breast milk combined with formula milk or other food. However, when we evaluated time of breastfeeding as an effect modifier, we observed similar results for the non-breastfed infants, which endows the study with greater consistency. One more potential limitation could be the fact that not all the serum samples were taken at exactly the same gestational age. In any case, even though it has been observed that Se decreases with gestational age (Pieczyńska and Grajeta, 2015), no significant association between Se and gestational age was found in our data, and the estimated change of mean Se for the observed period is negligible. Therefore, gestational age did not need to be included as one of the potential confounders of any of the models.

The main strength of this study is its prospective design, which made it possible to obtain detailed information concerning maternal and child sociodemographic, dietary and life style characteristics that may affect neuropsychological development, and also to continue with both exposure and neurodevelopment assessment at older ages.

As a conclusion, we observed a non-linear relationship between maternal Se concentrations and child neuropsychological development assessed at 5 years of age. Children whose mothers had Se concentrations above 85 μg/L at the first trimester of pregnancy obtained worst scores in the verbal and global memory scales. The similar relationship observed when neuropsychological development was assessed at 12 months of age, shows consistency in the results. Se concentrations in this population were within the range considered to be safe for human health, what suggests that prenatal and early life periods could be especially vulnerable to Se neurotoxicity; however further studies should be necessary in order to confirm these results.

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Figure captions

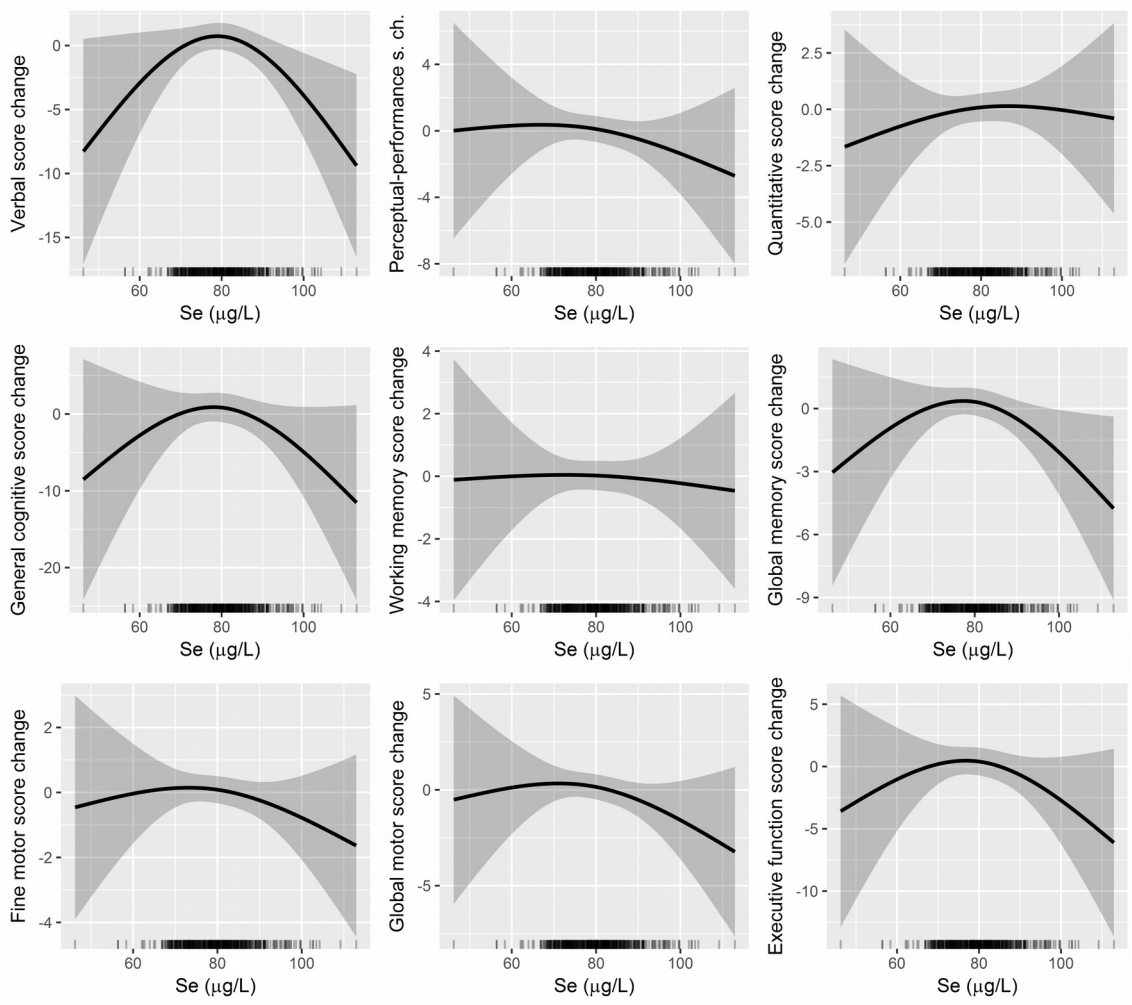

Fig. 1 Non-linear models for the association between maternal Se concentrations (measured at 12th week of gestation) and child neuropsychological development at 5 years of age. INMA-Valencia Project (2003-2012)

Footnote:

s.: score

Ch.: change

All models adjusted by sex, age at evaluation and psychologist
Verbal model additionally adjusted by maternal age, maternal country of birth, maternal educational level, parity, type of zone, maternal working status during pregnancy and BMI before pregnancy. Perceptual-performance model additionally adjusted by maternal age, maternal educational level, type of zone, smoking during pregnancy, parity, breastfeeding, maternal smoking at evaluation and maternal working status at evaluation. Quantitative model additionally adjusted by parental educational level, type of zone, maternal intelligence, parity and maternal age. General cognitive model additionally adjusted by maternal education, parity, type of zone, maternal age, paternal working status at evaluation, maternal intelligence and seafood intake during pregnancy. Working memory model

additionally adjusted by maternal country of birth, paternal educational level, parity, maternal smoking at evaluation and maternal intelligence. Global memory model additionally adjusted by maternal educational level, parity, type of zone, maternal intelligence, maternal country of birth, maternal age and seafood intake during pregnancy. Fine motor model additional adjusted by breastfeeding, maternal educational level, parity, type of zone and maternal smoking at evaluation. Global motor model additionally adjusted by maternal educational level and breastfeeding. Executive model additionally adjusted by maternal country of birth, maternal age, parental educational level, parity, type of zone, maternal smoking at evaluation and maternal intelligence

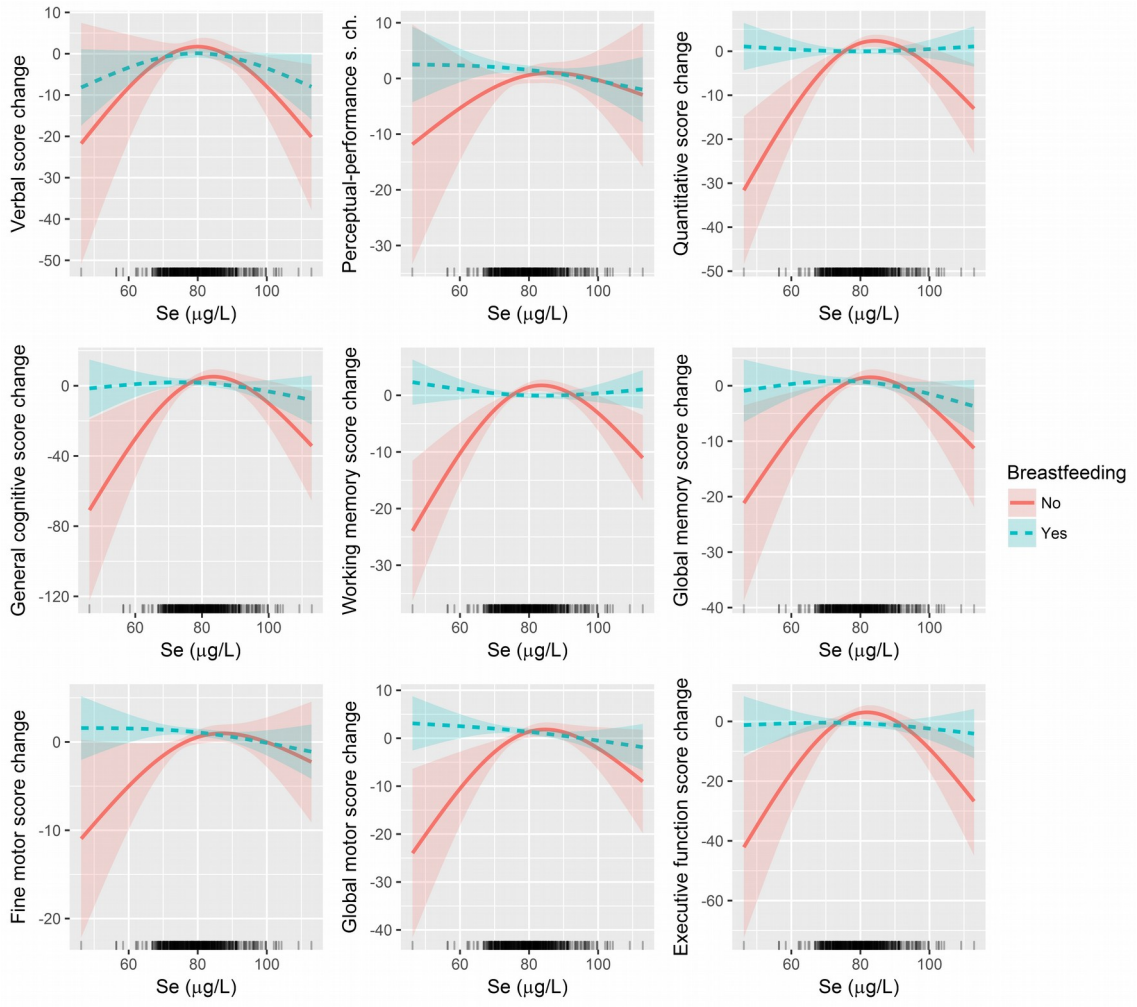

Fig. 2 Non-linear models for the association between maternal Se levels and child neuropsychological development according to breastfeeding. INMA-Valencia Project (2003-2012)

Verbal model additionally adjusted by maternal age, maternal country of birth, maternal educational level, parity, type of zone, maternal working status during pregnancy and BMI before pregnancy. Perceptual-performance model additionally adjusted by maternal age, maternal educational level, type of zone, smoking during pregnancy, parity, breastfeeding, maternal smoking at evaluation and maternal working status at evaluation. Quantitative model additionally adjusted by parental educational level, type of zone, maternal intelligence, parity and maternal age. General cognitive model additionally adjusted by maternal education, parity, type of zone, maternal age, paternal working status at evaluation, maternal intelligence and seafood intake during pregnancy. Working memory model additionally adjusted by maternal country of birth, paternal educational level, parity, maternal smoking at evaluation and maternal intelligence. Global memory model additionally adjusted by maternal educational level, parity, type of zone, maternal intelligence, maternal country of birth, maternal age and seafood intake during pregnancy. Fine motor model additional adjusted by breastfeeding, maternal educational level, parity, type of zone and maternal smoking at evaluation. Global motor model additionally adjusted by maternal educational level and breastfeeding. Executive

model additionally adjusted by maternal country of birth, maternal age, parental educational level, parity, type of zone, maternal smoking at evaluation and maternal intelligence

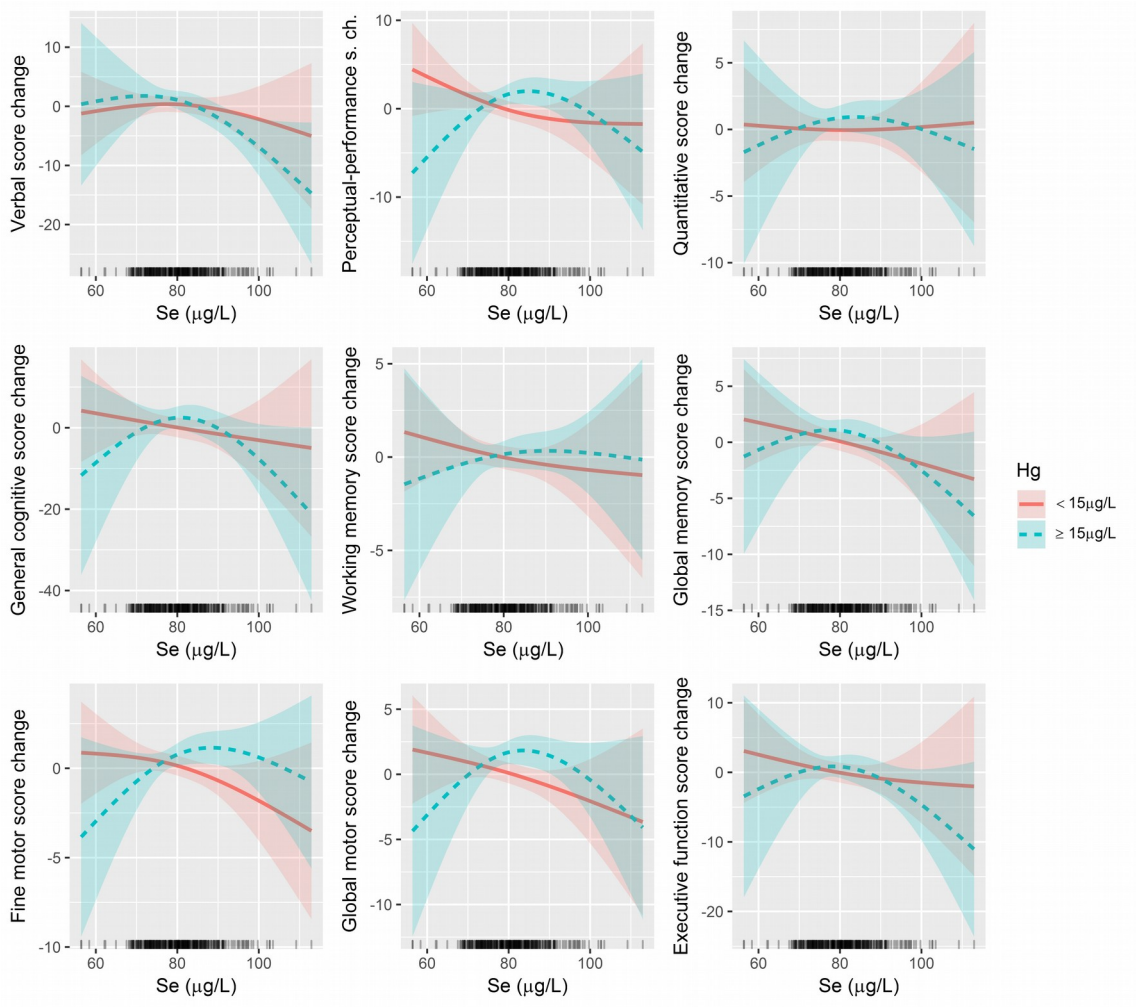

Fig. 3 Non-linear models for the association between maternal Se levels and child neuropsychological development according to cord blood mercury (<15 vs. ≥15µg/L). INMA-Valencia Project (2003-2012)

Verbal model additionally adjusted by maternal age, maternal country of birth, maternal educational level, parity, type of zone, maternal working status during pregnancy and BMI before pregnancy. Perceptual-performance model additionally adjusted by maternal age, maternal educational level, type of zone, smoking during pregnancy, parity, breastfeeding, maternal smoking at evaluation and maternal working status at evaluation. Quantitative model additionally adjusted by parental educational level, type of zone, maternal intelligence, parity and maternal age. General cognitive model additionally adjusted by maternal education, parity, type of zone, maternal age, paternal working status at evaluation, maternal intelligence and seafood intake during pregnancy. Working memory model additionally adjusted by maternal country of birth, paternal educational level, parity, maternal smoking at evaluation

and maternal intelligence. Global memory model additionally adjusted by maternal educational level, parity, type of zone, maternal intelligence, maternal country of birth, maternal age and seafood intake during pregnancy. Fine motor model additional adjusted by breastfeeding, maternal educational level, parity, type of zone and maternal smoking at evaluation. Global motor model additionally adjusted by maternal educational level and breastfeeding. Executive model additionally adjusted by maternal country of birth, maternal age, parental educational level, parity, type of zone, maternal smoking at evaluation and maternal intelligence

Table 1 Parental and child sociodemographic, environmental and dietary characteristics. INMA Project (Valencia, Spain, 2003-2012)

	N (%)	Mean (SD)
Maternal age, years		
< 25	37 (7.6)	74.6 (5.4)
25 - 29	165 (33.7)	79.2 (7.5)
30 - 34	207 (42.2)	80.7 (8.4)
≥ 35	81 (16.5)	81.6 (8.7)
Maternal BMI, kg/m^2		
< 18.5	16 (3.3)	78.7 (7.0)
18.5 - 25	330 (67.3)	79.7 (8.2)
25 - 30	98 (20.0)	81.4 (8.1)
≥ 30	46 (9.4)	78.8 (7.6)
Maternal country of birth		
Spain	454 (92.7)	80.3 (8.1)
Other	36 (7.3)	75.6 (6.8)
Parity		
0	267 (54.5)	80.0 (8.3)
1	189 (38.6)	79.9 (8.2)
≥ 2	34 (6.9)	79.5 (7.0)
Mother smoking at the beginning of pregnancy		
No	303 (61.8)	80.4 (8.5)
Yes	187 (38.2)	79.2 (7.3)
Maternal smoking habit in the presence of the child at 5 yrs		
Often	33 (6.9)	78.8 (8.7)
Occasionally	37 (7.7)	80.0 (7.0)
Rarely	27 (5.6)	80.4 (6.9)
Never	381 (79.7)	79.9 (8.3)
Maternal level of education		
Up to primary studies	138 (28.2)	79.3 (7.7)
Secondary studies	213 (43.5)	79.8 (7.8)
University	139 (28.4)	80.6 (9.0)
Paternal level of education		
Up to primary studies	216 (44.2)	79.4 (7.3)
Secondary studies	190 (38.9)	80.4 (9.0)
University	83 (17.0)	80.2 (8.1)
Parental social class		
Class I+II	133 (27.1)	80.1 (7.2)
Class III	137 (28.0)	79.1 (8.3)
Class IV+V	220 (44.9)	78.5 (8.2)
Maternal verbal IQ proxy at 5 yrs	9.86 ± 3.23[a]	
Maternal working status during pregnancy		
Non-worker	75 (15.3)	79.9 (7.6)
Worker	415 (84.7)	79.9 (8.2)
Maternal working status at 5 yrs		
Non-worker	145 (29.7)	79.3 (7.0)

Worker	344 (70.3)	80.2 (8.6)
Paternal working status at 5 yrs		
Non-worker	70 (14.5)	80.1 (8.5)
Worker	414 (85.5)	79.9 (8.1)
Area of residence		
Urban	50 (10.2)	80.8 (8.8)
Metropolitan	226 (46.1)	80.0 (8.0)
Semi-urban	180 (36.7)	79.9 (8.0)
Rural	34 (6.9)	78.1 (8.4)
Season of sampling		
Spring	158 (32.2)	80.6 (7.9)
Summer	128 (26.1)	78.8 (7.8)
Autumn	85 (17.3)	79.0 (8.5)
Winter	119 (24.3)	80.7 (8.3)
Seafood intake (g/day)	74.75 ± 35.32[a]	
Eggs intake (g/day)	19.35 ± 9.28[a]	
Bread intake (g/day)	87.76 ± 50.13[a]	
Child's sex		
Girl	238 (48.6)	79.7 (7.7)
Boy	252 (51.4)	80.1 (8.6)
Breastfeeding		
No	79 (16.2)	79.9 (8.6)
Yes	408 (83.8)	79.9 (8.0)
Child's age at MSCA administration	5.77 (0.16)	
TOTAL	490 (100)	79.9 (8.1)

BMI: body mass index before pregnancy
IQ: Intelligence quotient
Yrs : years
[a]Mean ± standard deviation
Class I+II: managerial jobs, senior technical staff and commercial managers; class III: skilled non-manual workers; and class IV+V: manual and unskilled workers

Table 2 Multivariate linear regression analysis between maternal Se concentrations and the mental scores for the McCarthy Scales of Children's Abilities at 5 years of age with and without interaction with breastfeeding, sex, and total mercury level

	Main Se effect beta (95% CI), p-val	**Se*Breastfeeding p-val**	**Se*Sex p-val**	**Se*THg p-val**
Verbal	-0.052 (-0.164, 0.061), 0.366	0.347	0.794	0.054
Perceptual-performance	-0.046 (-0.128, 0.036), 0.268	0.189	0.574	0.228
Quantitative	0.015 (-0.050, 0.080), 0.653	0.343	0.187	0.969
General cognitive	-0.085 (-0.283, 0.112), 0.395	0.415	0.697	0.759
Working memory	-0.006 (-0.054, 0.042), 0.793	0.357	0.331	0.293
Global memory	-0.041 (-0.110, 0.027), 0.234	0.507	0.721	0.809
Fine motor	-0.022 (-0.064, 0.021), 0.317	0.038	0.373	0.052
Global motor	-0.048 (-0.115, 0.019), 0.160	0.070	0.581	0.351
Executive function	-0.057 (-0.175, 0.060), 0.338	0.881	0.840	0.649

p-val: p-value

THg: total mercury. Categorized as <15 vs ≥15 µg/L according to the equivalent for the WHO Provisional Tolerable Weekly Intake (1.6 µg/kg of body weight per week)
All models adjusted by sex, age at evaluation and psychologist
Verbal model additionally adjusted by maternal age, maternal country of birth, maternal educational level, parity, type of zone, maternal working status during pregnancy and BMI before pregnancy
Perceptual-performance model additionally adjusted by maternal age, maternal educational level, type of zone, smoking during pregnancy, parity, breastfeeding, maternal smoking at evaluation and maternal working status at evaluation
Quantitative model additionally adjusted by parental educational level, type of zone, maternal intelligence, parity and maternal age
General cognitive model additionally adjusted by maternal education, parity, type of zone, maternal age, paternal working status at evaluation, maternal intelligence and seafood intake during pregnancy
Working memory model additionally adjusted by maternal country of birth, paternal educational level, parity, maternal smoking at evaluation and maternal intelligence
Global memory model additionally adjusted by maternal educational level, parity, type of zone, maternal intelligence, maternal country of birth, maternal age and seafood intake during pregnancy
Fine motor model additional adjusted by breastfeeding, maternal educational level, parity, type of zone and maternal smoking at evaluation
Global motor model additionally adjusted by maternal educational level and breastfeeding
Executive model additionally adjusted by maternal country of birth, maternal age, parental educational level, parity, type of zone, maternal smoking at evaluation and maternal intelligence